Structure-based exploration of the ganglioside GM1 binding sites of Escherichia coli heat-labile enterotoxin and cholera toxin for the discovery of receptor antagonists.

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Ganglioside GM1 is the natural receptor for cholera toxin (CT) and heat-labile enterotoxin (LT), which are the causative agents of cholera and traveler's diarrhea, respectively. This observation suggests that small molecules interfering with this recognition process may prevent entry of the toxins into intestinal cells, thereby averting their devastating effects. Here, the terminal sugar of ganglioside GM1, galactose, was chosen as a lead in designing such receptor antagonists. Guided by the experimentally determined binding mode of galactose, we selected a "substructure" for searching the Available Chemicals Database, which led to the purchase of 35 galactose derivatives. Initial screening of these compounds in an LT ELISA revealed that 22 of them have a higher affinity for LT than galactose itself. A structurally diverse subset of these galactose derivatives was selected for determination of IC50 values in the LT ELISA and IC50 values in a CT assay, as well as for the determination of Kd's using the intrinsic fluorescence of LT. The best receptor antagonist found in this study was m-nitrophenyl alpha-galactoside with an IC50 of 0.6 (2) mM in the LT ELISA and 0.72 (4) mM in the CT assay, 100-fold lower than both IC50 values of galactose. Careful analysis of our binding data and comparison with crystal structures led to the derivation of correlations between the structure and affinity of the galactose derivatives. These characteristics will be used in the design of a second round of LT and CT receptor antagonists.

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Structural basis for differential receptor binding of cholera and Escherichia coli heat-labile toxins: influence of heterologous amino acid substitutions in the cholera B-subunit.

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The closely related B-subunits of cholera toxin (CTB) and Escherichia coli heat-labile enterotoxin (LTB) both bind strongly to GM1 ganglioside receptors but LTB can also bind to additional glycolipids and glycoproteins. A number of mutant CT B-subunits were generated by substituting CTB amino acids with those at the corresponding positions in LTB. These were used to investigate the influence of specific residues on receptor-binding specificity. A mutated CTB protein containing the first 25 residues of LTB in combination with LTB residues at positions 94 and 95, bound to the same extent as native LTB to both delipidized rabbit intestinal cell membranes, complex glycosphingolipids (polyglycosylceramides) and neolactotetraosylceramide, but not to non-GM1 intestinal glycosphingolipids. In contrast, when LTB amino acid substitutions in the 1-25 region were combined with those in the 75-83 region, a binding as strong as that of LTB to intestinal glycosphingolipids was observed. In addition, a mutant LTB with a single Gly-33-->Asp substitution that completely lacked affinity for both GM1 and non-GM1 glycosphingolipids could still bind to receptors in the intestinal cell membranes and to polyglycosylceramides. We conclude that the extra, non-GM1 receptors for LTB consist of both sialylated and non-sialylated glycoconjugates, and that the binding to either class of receptors is influenced by different amino acid residues within the protein.

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Binding of cholera toxin B-subunits to derivatives of the natural ganglioside receptor, GM1.

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In a previous paper we showed that the B-pentamer of cholera toxin (CT-B) binds with reduced binding strength to different C(1) derivatives of N-acetylneuraminic acid (NeuAc) of the natural receptor ganglioside, GM1. We have now extended these results to encompass two large amide derivatives, butylamide and cyclohexylmethylamide, using an assay in which the glycosphingolipids are adsorbed on hydrophobic PVDF membranes. The latter derivative showed an affinity approximately equal to that earlier found for benzylamide (approximately 0.01 relative to native GM1) whereas the former revealed a approximately tenfold further reduction in affinity. Another derivative with a charged C(1)-amide group, aminopropylamide, was not bound by the toxin. Toxin binding to C(7) derivatives was reduced by about 50% compared with the native ganglioside. Molecular modeling of C(1) and C(7) derivatives in complex with CT-B gave a structural rationale for the observed differences in the relative affinities of the various derivatives. Loss of or altered hydrogen bond interactions involving the water molecules bridging the sialic acid to the protein was found to be the major cause for the observed drop in CT-B affinity in the smaller derivatives, while in the bulkier derivatives, hydrophobic interactions with the protein were found to partly compensate for these losses.

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1: *J Biochem (Tokyo)* 1994 Dec;116(6):1269-74 Related Articles, Books

On the role of the carboxyl group of sialic acid in binding of cholera toxin to the receptor glycosphingolipid, GM1.

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The carboxyl group of the natural cholera toxin receptor, the ganglioside GM1, Gal beta 1-3GalNAc beta 1-4(NeuAc alpha 2-3)Gal beta 1-4Glc beta 1-Cer, has been converted to a number of C(1)-amides of NeuAc. The binding of cholera toxin B-subunit to these derivatives was monitored by exposing the modified glycolipids, on solid phases, to radiolabeled toxin. Binding was obtained, although substantially reduced, with the amide and to a lesser extent with the benzylamide and also the C(1)-alcohol. In the assay system used, the methyl-, ethyl-, or propylamides did not bind. It was concluded that the hydrogen bonding capacity of a carboxyl or amide group is needed for strong binding. This is in agreement with the recently published crystal structure of the B-subunit in complex with the GM1 pentasaccharide.

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Structural foundation for the design of receptor antagonists targeting Escherichia coli heat-labile enterotoxin.

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BACKGROUND: Escherichia coli heat-labile enterotoxin (LT) is the causative agent of traveller's diarrhoea, and it is also responsible for the deaths of hundreds of thousands of children per year in developing countries. LT is highly homologous in sequence, structure and function to cholera toxin (CT). Both toxins attack intestinal epithelial cells via specific binding to the branched pentasaccharide of ganglioside GM1 at the cell surface. A receptor-binding antagonist which blocked this interaction would potentially constitute a prophylactic drug conferring protection both against the severe effects of cholera itself and against the milder but more common disease caused by LT. RESULTS: Four derivatives of the simple sugar galactose, members of a larger series of receptor antagonists identified by computer modeling and competitive binding studies, have been co-crystallized with either the full LT AB5 holotoxin or the LT B pentamer. These crystal structures have provided detailed views of the toxin in complex with each of the four antagonists: melibionic acid at 2.8 A resolution, lactulose at 2.65 A resolution, metanitrophenylgalactoside (MNPG) at 2.2 A resolution and thiodigalactoside (TDG) at 1.7 A resolution. The binding mode of each galactose derivative was observed 5-15 times, depending on the number of crystallographically independent toxin B pentamers per asymmetric unit. There is a remarkable consistency, with one important exception, in the location and hydrogen-bonding involvement of well-ordered water molecules at the receptor-binding site. CONCLUSIONS: The bound conformations of these receptor antagonist compounds preserve the toxin-galactose interactions previously observed for toxin-sugar complexes, but gain additional favorable interactions. The highest affinity compound, MNPG, is notable in that it displaces a water molecule that is observed to be well-ordered in all other previous and current crystal structures of toxin-sugar complexes. This could be a favorable entropic factor contributing to the increased affinity. The highest affinity members of the present set of antagonists (MNPG and TDG) bury roughly half (400 A2) of

the binding-site surface covered by the full receptor GM1 pentasaccharide, despite being considerably smaller. This provides an encouraging basis for the creation of subsequent generations of derived compounds that can compete effectively with the natural receptor.

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